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09/646,740

TITLE: ISOFLAVONOIDS FROM IRIS-CROCEA.
AUTHOR(S): SHAWL A S; KUMAR T
SOURCE: **PHYTOCHEMISTRY (OXF), (1992) 31 (4), 1399-1401.**

TITLE: Determination of isoflavonoid glucosides in rhizomes of ***Belamcanda*** chinensis by high performance liquid chromatography.
AUTHOR: Cui J.M.; Chung H.S.; Woo W.S.

Korean J of Phamacognosy (1993), 24/4 (309-312).

SOURCE: TITLE: Studies on chemical constituents of ***Belamcanda*** chinensis (L.)DC. II
AUTHOR(S): Zhou, Li Xin; Lin, Mao
SOURCE: **Chin. Chem. Lett. (1997), 8(2), 133-134**

TITLE: Pharmacological studies of ***tectoridin*** and ***tectorigenin***
AUTHOR(S): Esaki, Shunji
SOURCE: **Nippon Yakurigaku Zasshi (1968), 64(2), 186-98**

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Kor. J. Pharmacogn.
24(4):309~312(1993)

Determination of Isoflavonoid Glucosides in Rhizomes of *Belamcanda chinensis* by High Performance Liquid Chromatography

Jiong Mo Cui*, Ha Sook Chung and Won Sick Woo

*Department of Pharmacognosy, Yanbian Medical College, Yanji, China and
Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea

Abstract—A new method for separation and quantitative determination of isoflavone glucosides in rhizomes of *Belamcanda chinensis* (Iridaceae) by high performance liquid chromatography was elaborated. A reverse-phase system with a Spheri-5 RP-18 column using MeOH : HOAc : H_2O (24 : 5 : 71) as a mobile phase was developed. The isoflavonoids were detected at 268 nm and the analysis was successfully carried out within 15 min.

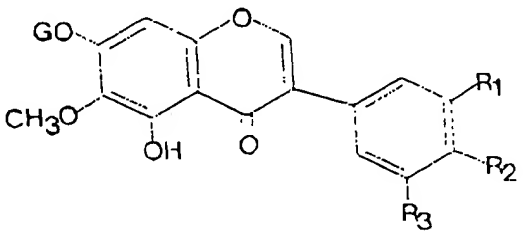
Keywords—*Belamcanda chinensis* · Iridaceae · isoflavonoid glucosides · iridin · tectoridin · HPLC

The rhizomes of *Belamcanda chinensis* (Iridaceae) have been used as a Chinese drug for the treatment throat troubles such as tonsillitis[1].

In the previous study, we examined the chemical constituents of this plant in order to isolate its active principles and to determine the structures of two major isoflavonoid glucosides such as iridin and tectoridin[2] together with a number of minor constituents[3~5].

Recently, Wu and Xu reported an analytical method of tectoridin in *B. chinensis* by square wave voltametry[6]. The present study describes a new method for separation of iridin and tectoridin and their simultaneous quantitative analysis by high performance liquid chromatography (HPLC).

According to our experiences, a reverse-phase column with a polar elution solvent mixture as a separation system has been established. It was revealed that major components in this plant, iridin and tectoridin (Fig. 1) could be separated with reasonable accuracy and this method may be employed for the estimation of flavone glucosides in pharmaceutical preparations of rhizomes of *B. chinensis*.



	R_1	R_2	R_3
1	OH	OCH_3	OCH_3
2	H	OH	H

Fig. 1. Structure of iridin(1) and tectoridin(2)

Results and Discussion

Selection of separation system

The rhizomes of *B. chinensis* were extracted

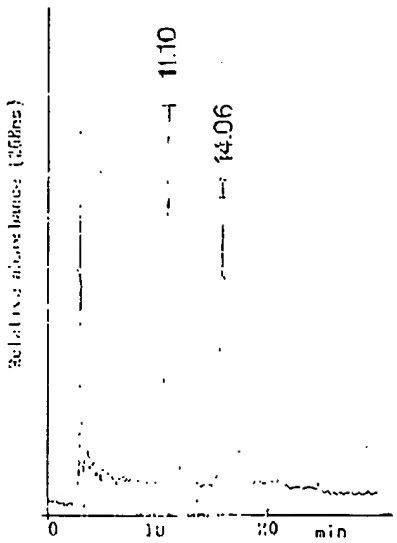


Fig. 2. HPLC chromatogram of the standard isoflavonoids mixture Iridin(I); Tectoridin(T)
Analytical conditions; column, Spheri-5 RP-18, 5 mμ, 4.6 mmI.D. ×220 mm; eluent, MeOH : HOAc : H_2O(24 : 5 : 71); flow rate, 1.0 ml/min; chart speed. 1.5 cm/min; detector, UV(268 nm).
Concn.; 5.0 μg/ml

with MeOH and the methanolic extract thus obtained was subjected to HPLC for iridin and tectoridin, monitoring with various solvent mixtures in order to find a good separation system. A reverse-phase system with a Spheri-5 RP-18 column using MeOH : HOAc : H_2O(24 : 5 : 71) as a mobile phase was found to be applicable to the analysis of isoflavonoids in *B. chinensis*. The isoflavonoids were detected at 268 nm by UV detector.

Fig. 2 shows the HPLC chromatogram of a mixture of pure two isoflavonoids, which was obtained by elution with this solvent system. It can be seen that the baseline separation of the peaks obtained was relatively good.

Various parameters such as capacity factor of column, relative retention, resolution and retention time for the analytical conditions were calculated from the chromatogram(Fig. 2) and indicated in Table I.

Fig. 3 shows the typical HPLC chromatograms of the methanolic extracts in the rhizomes of *B. chinensis*, domestics and imports from China. As shown in the chromatograms, the peaks appearing as high intensities are iridin(I) and tectoridin(T). It was evident that two compounds are major components of *B. chinensis* and solvent mixture and analytical conditions are very excellent in the chromatograms.

Table I. HPLC parameters for isoflavonoids

Compounds	t_R	t_R'	k′	α	R
Tectoridin	11.10	8.81	3.85	1.47	4.39
Iridin	14.06	11.77	5.65		

t_R; retention time, t_R'; net retention time,
k′; capacity factor of column,
α; relative retention, R; resolution. t_0=2.29 min
Solvent mixture; MeOH : HOAc : H_2O(24 : 5 : 71), flow rate; 1.0 ml/min.

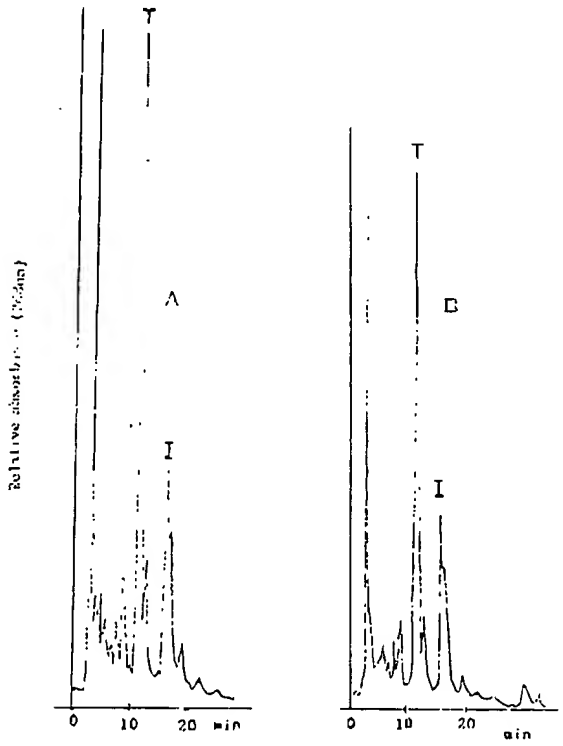


Fig. 3. HPLC chromatogram of methanol extract from domestic(A) and China(B) samples
Iridin(I); Tectoridin(T)
The analytical conditions were the same as those in Fig. 2.
Concn.; A: 2.0 μg/10 ml; B: 0.25 μg/10 ml

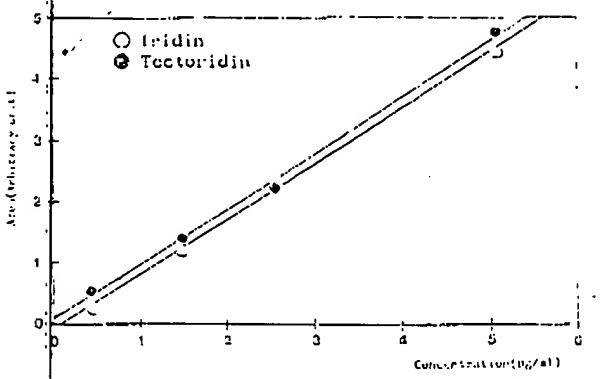


Fig. 4. Calibration curves for isoflavonoids

Calibration curves

Based on chromatograms of varying amounts of pure isoflavonoids obtained by the reverse-phase system, their individual calibration curves were composed and shown in Fig. 4. The regression equation was calculated and statistical analysis was carried out to check for linearity of separation system.

Iridin: $Y_1=90393X_1-8474$ (correlation coefficient, $r_1=0.991$);

Tectoridin: $Y_2=90727X_2+7127$ (correlation coefficient, $r_2=0.998$).

Each isoflavonoid component had a linear relationship between the amount ($\mu g=X_1, X_2$) and peak area (Y_1, Y_2). All the correlation coefficients of two calibration curves lay in the vicinity of the value of 1.00.

Determination of isoflavonoids in the rhizomes of *B. chinensis*

A definite amount of the rhizomes of *B. chinensis* was pretreated by the method described in the experimental section and the content of isoflavonoids was analysed by the reverse-phase system. It was revealed that the contents of iridin and tectoridin in the rhizomes collected in Korea were analysed to be 0.3535% and 1.2140%, respectively, where as 0.4029% and 1.5350% in that imported from China. The method can be used to confirm isoflavonoids of *B. chinensis* not only quantitative but qualitative analysis.

Experimental

Plant material—The rhizomes of *B. chinensis* were collected in the vicinity of Seoul. The Chinese origin was perchased from a drug store in Seoul and botanically identified.

Apparatus—The apparatus used was as follows: HPLC, Spectra Physics, Inc. attached with Spectra 100 variable wavelength detector; Injector, Rheodyne injection valve(10 μl) for HPLC; Column, Spheri-5 RP-18, dry-packed in 4.6 mm ID×220 mm capillary column; Pump, SP 8800 ternary HPLC pump; Integrator, SP 4270 integrator.

Reagents—Reagents and extraction solvents were first grade.

Iridin, mp 208° and tectoridin, mp 257~258° were isolated from the rhizomes of *B. chinensis* according to the method of Lee, *et al.*[2]

Chromatographic conditions—As a mobile phase, MeOH:HOAc:H_2O(24:5:71) in reverse-phase system was used. The analysis was carried out at room temperature in flow rate of 1.0 ml/min. The substances eluted were detected by UV detector at wavelength of 268 nm. The chart speed was 1.5 cm/min.

Preparation of calibration curves—Each isoflavonoids(10 mg) was dissolved in 25 ml of MeOH. These solutions were further diluted stepwise with MeOH solution to 0.625, 1.25, 2.5 and 5.0 μg/ml. And 10 μl of each solution was injected on the column. The calibration curves were depicted by plotting the amounts of samples against peak areas.

Analysis procedure—Two grams of accurately weighed were extracted three times with 25 ml of boiling MeOH for 3 hr on a water bath and filtered. The methanolic solution was concentrated to dryness and lyophilized. The extract (10 mg) was dissolved in 25 ml of MeOH and diluted 0.25, 0.1 and 0.5 μg/10 ml. And 10 μl

of each solution was applied and eluted to obtain the chromatogram.

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